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Determination of physicochemical properties of capsaicin and cytotoxic effect of capsicum extract in breast cancer (MCF7) cell line

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Abstract

Capsaicin (Trans -8-methillyl-6-nonamide) the major pungent ingredient in genus Capsicum, has long been used in food additives and drugs. Capsaicin has been shown to inhibit growth and induce apoptpsis in various transformed cell types in vitro, but not in normal counterpart cells.we investigated about capsaicin in two experimental and computational studies. The medicinal plants were collected, powdered, extracted, lyophilized and kept in -20° C. Breast cancer (MCF7) cell lines and normal connective tissue cell line (L929)were cultured in DMEM medium. Capsicum was extracted; and different dilutions of capsicum extract (1.60 to 1.200) were added to cell culture. Cell viability was quantitated by MTT assay after 24, 48 and 72 hours. The effects of capsicum extract on cell viability were observed after 48 hours on cell lines.capsicum extract doses in dilution 1.100 and 1.70 inhibited 50% cell growth (IC50) in MCF7 cell line after 48hours of incubation, respectively Our study shows that capsicum fresh extract has cytotoxic effects on tumor cells. In this article, theoretical methods have been used for calculation of physical parameters in polyphenols compounds in extract of *capsicum*. We calculated physical parameters like atomic charges, energy (ΔE), NMR determinan and distance matrix determinant, and in this work we used Gaussian 03at NMR by using HF methods with 3-21Gbasis set.

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Introduction

Capsaicin, the major pungent in gredient in genus Capsicum, has long been used in food additives and drugs. Capsaicin has been shown to inhibit growth and induce apoptosis in various transformed cell types in vitro, but not in normal counterpart cells (Ji-Seon *et al.,* 2004).Capsaicin, a major ingredient of hot pepper, was considered to exhibit an anti-inflammatory property. In order to clarify the signalling mechanism underlying the anti-inflammatory action of capsaicin, Chu-Sook Kim and *et al* investigated the effect of capsaicin on the production of inflammatory molecules in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages. The level of PGE2 was measured by EIA. Significant inhibition of the production of LPS-induced PGE2 by capsaicin was observed in a dose-dependent manner (Chu-Sook *et al.,* 2003).There has been a substantial body of data, supporting that dietary factors have a profound impact on prevention as well as etiology of human cancer. Capsaicin has been tested by many investigators for its effects on experimental carcinogenesis and mutagenesis. Data in the literature indicate that capsaicin has dual effects on carcinogenic and mutagenic processes. At present, there is no solid evidence that hot red and chili peppers or their principal pungent ingredient capsaicin are carcinogenic in humans although results of early investigations with experimental animals exhibit the moderate tumorigenicity of this compound. In contrast, recent studies reveal substantial antigenotoxic and anticarcinogenic effects of capsaicin, suggesting this compound as another important dietary phytochemical with a potential chemopreventive activity. Some pungent constituents present in ginger and other zingiberaceous plants have potent antioxidant and anti-inflammatory effects, and some of them exhibit anti-tumor promotional activity in experimental carcinogenesis (Young-Joon *et al.*, 1998).In Asia, nontoxic dietary products are considered desirable primary prevention vehicles for conquering cancer. As early as 1978, investigators in Korea carried out extensive long-term anticarcinogenicity experiments using the mouse lung tumor model and observed an anticarcinogenic effect of Panax ginseng C.A. Meyer extract in 1980. The results showed that natural products can provide hope for human cancer prevention. A newly established nine-week medium-term model using mouse lung tumors (Yun's Model) could confirm the anticarcinogenicity of ginseng that varies according to its type and age(Taik-koo, 1999).Capsaicin (trans-8-methyl-*N*-vanillyl-6-nonenamide), a natural product of Capsicum species, is known to induce excitation of nociceptive terminals involved in pain perception. Recent studies have also shown that capsaicin not only has chemopreventive properties against certain carcinogens and mutagens but also exerts anticancer activity(Jeong-Ki Mm, 2004).Lung cancer is a serious health problem in most developed countries and its incidence rate is profusely increasing. Capsaicin, a component of red chilli and red pepper has been studied widely for its chemopreventive properties (Anandakumar *et al.*, 2012). Capsicum (chilli) peppers are widely used as a spice. Capsicum is listed by the Council of Europe as a natural source of food flavouring (category N2). This category indicates that capsicum can be added to foodstuffs in small quantities, with a possiblelimitation of an active principle (as yet unspecified) in the final product. Previously, capsicum has been stated to be GRAS (Generally Recognised As Safe). Capsaicin has effects on nervous, cardiovascular, respiratory, thermoregulatory and gastrointestinal systems .Capsaicin has been used as a neuro chemical tool for studying sensory neurotransmission (Locock, 1985).In vitro and animal studies Infusion of capsaicin (200 mg/kg, by intravenous injection) has been reported to evoke dose-dependent catecholamine secretion (adrenaline, noradrenaline) from the adrenal medulla of pentobarbitone-anaesthetised rats (Watanabe, 1987).The addition of capsaicin (0.014%) to a high-fat (30%) diet fed to rats was found to reduce serum-triglyceride concentrations but to have no effect on serum cholesterol or pre-b-lipoprotein concentration. Capsaicin was thought to stimulate lipid mobilization from adipose tissue. Lipid absorption was unaffected by capsaicin supplementation. Activities of two

hepatic enzymes, glucose-6-phosphate de hydrogenase and adipose lipoprotein lipase, were elevated in rats when capsaicin was added to the diet (Kawada, 1986). Capsicum extracts fed orally to hamsters have been reported to significantly decrease hepatic vitamin A concentrations. Serum vitamin A concentrationswere not affected (Agrawal, 1985). Both the gastric and duodenal mucosae are thought to contain'capsaicin-sensitive' areas which afford protection against acidand drug-induced ulcers when stimulated by hydrochloric acid or by capsaicin itself. Stimulation causes an increase in mucosal blood flow and/or vascular permeability, inhibits gastric motility, and activates duodenal motility. Desensitization of these areas, using a regimen involving subcutaneous or oral administration of capsaicin, is thought to remove the protection (Maggi *et al.*, 1987). However, capsaicin desensitization was found to have little effect on peripheral responses to stress (i.e. ulcer formation) but did enhance central responses (increase in plasma corticosterone concentration) in rats. The increase in plasma corticosterone concentration observed in capsaicin-desensitised rats was similar in stressed and non-stressed animals.Capsaicin was found to influence adrenal cortical activity independently of the presence of a stress factor and may represent a stressor in itself. Capsaicin desensitisation was not found to influence basal gastric acid secretion in non-stressed rats, but did lower pentagastrin-stimulated gastric output. However, other results have reported that capsaicin desensitisation does increase acid secretion.(Dugani *et al.*, 1986).

Material and method

Preparation of extracts

Thirty grammes of each sample were placed in a Soxhlet cartridge. Each test sample was extracted semi-continuously from the Soxhlet with methylene chloride (DCM) and methanol diluted with distilled water according to a ratio of 80:20 v/v. The residual marc from each sample was infused with distilled water for 5 hours and filtered using absorbent cotton. The filtrates obtained were centrifuged at 2000 rpm for 5 minutes. The organic extracts were concentrated under reduced pressure in the rotavapor and the aqueous extracts were dried in a ventilated oven at a temperature of 45-50 °C. The various extracts were stored in coloured flasks for phytochemical screening.

Cell culture

MCF7 cells was produced from Pasteur institute of Iran and were cultured in RPMI medium supplemented with 10% fetal bovine serum, 2 mg/ml sodium bicarbonate, 10mM HEPES, 100 unit/ml of penicillin ,and 100 µg/ml streptomycin at 37°C in humidified atmosphere with 5% CO_2. Cell suspension (5 x 10^5 cells/ml) was plated out into 96-well microtiter plate. Plant extracts were initially dissolved in DMSO as mentioned earlier, with the final concentration of DMSO being 0.1% (v/v). Serial dilutions of the sample were prepared in RPIM1640. The cytoxicity profiles of the extracts were assessed using 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) microculture tetrazolium viability assay as described by Mosmann . Thereafter, various concentrations of the plant extract samples were plated out in triplicates. Each plate included untreated cell controls and a blank cell-free control. After 68 h of incubation, MTT (5 µg/ml) was added to each well and re-incubated for further 4 h. Then, the media was removed and DMSO was added into each well to solubilize the formazan crystals. Finally, the absorbance was read at wavelength of 595 nm using a microtitre plate reader (Labsystems iEMS Reader MF) and the percentage cell viability was calculated with the appropriate controls taken into account. The concentration which inhibited 50% of cellular growth (IC_{50} value) was determined and the inhibitory rate of cell proliferation was calculated by the following formula

$$\text{Growth inhibition} = \frac{\text{ODcontrol} - \text{ODtreated}}{\text{ODcontrol}} \times 100$$

Where OD is the optical density.

Cytotoxicity of the sample towards the cancer cells was expressed as IC_{50} values (i.e. the EI extract concentration reducing the absorbance of treated cells by 50% in respect to untreated cells).

Computational method

An accurate knowledge of the magnitude of NMR tensors has been found to be valuable in indentifying biomolecular structure and dynamics with NMR spectroscopy (Dios *et al.*, 1996). Therefore, for reliable structural investigations, it is necessary to determine each nucleus of interest involved in the hydrogen-bonding network. Quantum chemical calculations are increasingly being used to rationalize the relationship between shielding tensors and biological structures (Monajjemi *et al*, 2008; Pecul Sadlej, 1998).

The following quantities are often used to describe NMR shielding tensors – namely, the isotropic, anisotropic shielding, and the asymmetry parameters:

a.The isotropic value (σ_{iso}) of the shielding tensor, which is defined as

$$\sigma iso = \frac{1}{2}(\sigma 11+\sigma 22+\sigma 33) \quad [1]$$

b. The anisotropy parameter ($\Delta\sigma$), which is defined as

$$\Delta\sigma = \sigma 33 - \frac{1}{2(\sigma 11 + \sigma 22)}$$

c. The asymmetry parameter (η), which is given.

$$\eta = \frac{\sigma 22 - \sigma 11}{\sigma 33 - \sigma iso}$$

Computational biology techniques play a key role in expanding our knowledge on the mode of behavior of solvents in normal biological processes. Physics-based computations such as biomolecular simulation simulate biomolecular motion according to the laws of physics; they provide quantitative information on biomolecular dynamics and energetics, as well as help with the interpretation of biophysical data (Diez *et al.*, 2006 and Autschbach, 2004)This research is an example of how quantum mechanical techniques can be successfully applied to biologically relevant problems in rather large and complex systems. For this purpose, the electronic structure calculations are performed on capsaicin using Gaussian 09 software. First, we considered the geometry optimizations of cap existing in, carrying out the Hartree–Fock level of theory using the 3–21G, and 6–31G basis set in the gas phase after optimization, the authors calculated NMR shielding parameters for some nuclei involved in the cap.

Result

Cell viability was quantitated by MTT assay after 24, 48 and 72 hours. The effects of capsicum extract on cell viability were observed after 48 hours on cell lines.capsicum extract doses in dilution 1.100 and 1.70 inhibited 50% cell growth (IC50) in MCF7 cell line after 48hours of incubation, respectively Our study shows that capsicum fresh extract has cytotoxic effects on tumor cells.

Table 1. Ohysical properties computed by chem bio office 2010

Capsaicin	Connoly Accessible Area	Connoly Molecular Area	Connoly solvent Exclude volume	Exact Mass	Mol Formula	Elemental Analysis
	676.148 Angstroms Squared	353.204 Angstroms Squared	307.257 Angstroms Cubed	305.1990937382 g/Mol	C18H27NO3	C, 70.79; H, 8.91; N, 4.59; O, 15.72

Table 2. Physico chemical properties computed by chem bio office 2010

Capsaicin	Freezing Point [p=1atm]	Critical Temperature	Critical Pressure	Critical Volume	Heat of Formation [T=298.15K, p=1atm]:	Gibbs Energy [T=298.15 K, p=1atm	Ideal gas thermal capacity for T= 298.15 [K] and p=1atm
	611.14 [K]	943.95 [K]	18.452 [bar]	971.50 [cm3/mol]	-499.59 [kJ/mol]	-37.460 [kJ/mol]	392.67

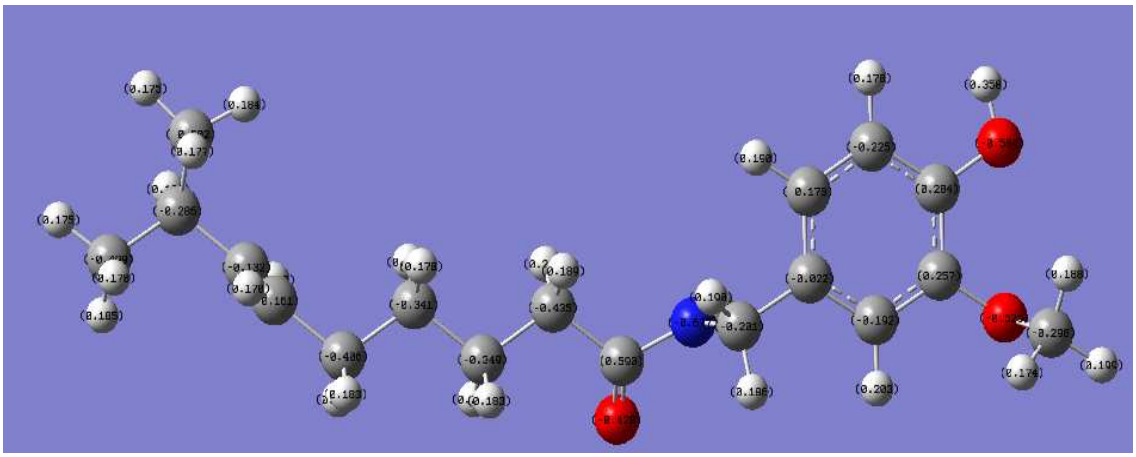

Fig 1. Capsaicin molecule

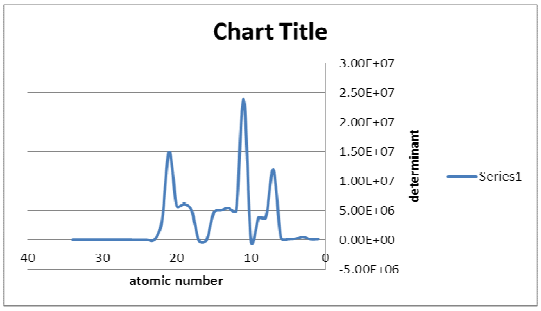

Fig 2. Isotropic determinant versus number of atom

Discussion

The use of herbs as complementary and alternative medicine has increased dramatically in the last 20–25 years. According to World Health Organization (WHO) traditional medicines are relied upon by 65–80% of the World's population for their primary health care needs. Moreover, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine. The beneficial health effects of many plants, used for centuries as seasoning agents in food and beverages, have been claimed for preventing food deterioration and as antimicrobials against pathogenic microorganisms. Antimicrobial potential of different medicinal plants is being extensively studied all over the world (Rios *et al.*, 2005; Chopra *et al.,* 1997).

Computational modeling can be a useful partner in biotechnology, in particular, in nanodevice engineering. Such modeling guides development through nanoscale views of biomolecules and devices not available through experimental imaging methods. We illustrate the role of computational modeling, mainly of molecular dynamics, through four case studies: development of silicon bionanodevices for single molecule electrical recording, development of carbon nano-tube-biomolecular systems as in vivo sensors, development of lipoprotein nanodiscs for assays of single membrane proteins, and engineering of oxygen tolerance into the enzyme hydrogenase for photosynthetic hydrogen gas production. The four case studies show how molecular dynamics approaches were adapted to the specific technical uses through (i) multi-scale extensions, (ii) fast quantum chemical force field evaluation, (iii) coarse graining, and (iv) novel sampling methods. The adapted molecular dynamics simulations provided key information on device behavior and revealed development opportunities, arguing that the "computational microscope" is an indispensable nanoengineering tool (Aksimentiev *et al.*, 2008).

An improved understanding of the nature of the biological interactions causing the shift in NMR or thermochemical parameters may hold the key to finding the most suitable condition for the occurrence of various biological phenomena – specifically, mutation. It seems likely that NMR chemical shielding tensors may play an even more important role in structural determination. It has been found

that hydrogen bonding is the most important factor for dishelming of the electronic charge density around noticed nuclei. Natural products from plants have been valuable sources for anticancer drug discovery (Pazdur *et al*., 2002).

The calculation of NMR parameters using semi-empirical and ab initio techniques has become a major and powerful tool in the investigation to look at how variations in the molecular structure occurs. The ability to quickly evaluate and correlate the magnitude and orientation of the chemical shielding anisotropy tensor with variations in bond length, bond angles and local coordination and nearest neighbor interactions has seen a number of recent applications in the investigation of molecular structure .The calculations also provide valuable information for exploring the experimental NMR chemical shifts with the molecular geometry and environment. Also NMR chemical shifts are quite sensitive to intermolecular interactions (Monajjemi *et al.,* 2008).

References

Agrawal RC. 1985. Chilli extract treatment and induction of eye lesions in hamsters. Toxicology Letters **28,** 1–7.
http://dx.doi.org/10.1016/0378-4274(85)90002-5

Aksimentiev A, Brunner R, Cohen J, Comer J, Cruz-Chu E, Hardy D. 2008. Computer modeling in biotechnology: a partner in development. Methods in Molecular Biology **474,** 181-234.
http://dx.doi.org/10.1007/978-1-59745-480-3_11

Anandakumar P, Kamaraj S, Jagan SM. 2012. Capsaicin inhibits benzo(a)pyrene-induced lung carcinogenesis in an in vivo mouse model. Inflammation Research. Epub ahead of print.
http://dx.doi.org/10.1007/s00011-012-0511-1

Autschbach J. 2004. The calculation of NMR parameters in transition metal complexes. In: Kaltsoyannis N, McGrady JE, editors. Principles and Applications of Density Functional Theory in Inorganic Chemistry I, Structure and Bonding. Heidelberg, Germany: Springer Verlag. **112,** 1–48.

Chopra I, Hodgson J, Metcalf B, Poste G. 1997. The search for antibacterial agents effective against bacteria resistant to multiple antibiotics. Antimicrobial Agents and Chemotherapy (AAC). **4,** 497–503.

Chu-Sook K, Teruo K, Byung-Sam K. 2003. Capsaicin exhibits anti-inflammatory property by inhibiting IkB-a degradation in LPS-stimulated peritoneal macrophages .cellular signaling. **3,** 299-306.

Diez NM, Senent ML, Garcia B. 2006. Ab initio study of solvent effects on the acetohydroxamic acid deprotonation processes. Journal of Chemical Physics. **324,** 350–358.

Dios AC, Oldfield E. 1996. Recent progress in understanding chemical shifts. Solid State Nuclear Magnetic Resonance. **6,** 101–125.
http://dx.doi.org/10.1016/0926-2040(95)01207-9

Dugani A, Glavin GB. 1986. Capsaicin effects on stress pathology and gastric acid secretion in rats. Life Sciences **39,** 1531–1538.
http://dx.doi.org/10.1016/0024-3205(86)90383-8

Jeong-Ki Mm. Capsaicin Inhibits in Vitro and in Vivo Angiogenesis . American Association for Cancer Research. 2004.

Ji-Seon L, Jong-Sun CH,JiYoul L, Jung-Ae K. 2004. Capsaicin -induced apoptosis and reduced release of reactive oxygen species in MBT -2 murine bladder tumor cells. Archives of Pharmacal Research. **27(11),** 1147-1153.
http://dx.doi.org/10.1007/BF02975121

Kawada T. 1986. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. Journal of Nutrition **116,** 1272–1278.

Locock RA. 1985. Capsicum. Canadian pharmacists journal **118,** 517–519.

Maggi CA. 1987. Capsaicin-sensitive mechanisms and experimentally induced duodenal ulcers in rats. Journal of Pharmacy and Pharmacology **39**, 559–561.
http://dx.doi.org/10.1111/j.2042-7158.1987.tb03179.x.

Monajjemi M , Rajaeian E, Mollaamin F, Naderi F, Saki S. 2008. Investigation of NMR shielding tensors in 1, 3 dipolar cycloadditions: solvents dielectric effect. Physics and Chemistry of Liquids **46(3),** 299–306

Monajjemi M, Haddadi A, Honarparvar B, Irani S, Mollaamin F, Tahan A. 2008. NMR and solvent effect study on the thymine-adenine-thymine sequence: a theoretical investigation on the chemical behavior of nucleotides in solution. Egyptian Journal of Biochemistry and Molecular Biology **26,** 83–100. http://dx.doi.org/10.4314/ejbmb.v26i1.35959

Pecul M, Sadlej J. 1998. 15N chemical shift tensor magnitude and orientation in the molecular frame of uracil determined via MAS NMR. Journal of Chemical Physics. **234,** 111–119.

Rios JL, Recio MC. 2005. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacolog. **100,** 80–84. http://dx.doi.org/10.1016/j.jep.2005.04.025

Schwartsmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N, Parkinson DR. 2002. Anticancer drug discovery and development throughout the world. Journal of Clinical Oncology (JCO). **20,** 47S–59S.

Taik-koo Y. 1999. Update from Asia: Asian Studies on Cancer Chemoprevention. Cancer prevention: Novel nutrient and pharmaceutical development. **889,** 157-192.

Watanabe T. 1987. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. Biochemical and Biophysical Research Communications. **142,** 259–264. http://dx.doi.org/10.1016/0006-291X(87)90479-7

Young-Joon S, Eunyong L. 1998. Chemoprotective properties of some pungent ingredients present in red pepper and ginger.Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. **402,** 259-267.